INDUCTION OF TUMOR IMMUNITY BY VARIANTS OF FOLATE BINDING PROTEIN

The present invention claims priority to U.S. Provisional Patent Application Ser. No. 60/274,676 filed Mar. 9, 2001, incorporated by reference herein in its entirety.

The government owns rights in the present invention pursuant to United States Army grant number DAMD 17-94-J-4313.

FIELD OF THE INVENTION

The present invention is directed to the fields of cancer and immunology. Specifically, the present invention is directed to compositions and methods for tumor vaccines directed to tumor antigens and is directed to specific epitopes on these antigens that are recognized by cytotoxic T-lymphocytes (CTL). More specifically, the present invention regards compositions and methods for variants of folate binding protein (FBP).

BACKGROUND OF THE INVENTION

Tumor reactive T-cells have been reported to mediate therapeutic responses against human cancers (Rosenberg et al., 1988). In certain instances, in human immunotherapy trials with tumor infiltrating lymphocytes (TIL) or tumor vaccines, these responses correlated either with in vitro cytotoxicity levels against autologous tumors (Aebersold et al., 1991) or with expression of certain HLA-A,B,C gene products (Marincola et al., 1992). Recent studies (Ioannides et al., 1992) have proposed that in addition to virally encoded and mutated oncogenes, overexpressed self-proteins may elicit some degree of tumor-reactive cytotoxic T-lymphocytes (CTLs) in patients with various malignancies (Ioannides et al., 1992; Ioannides et al., 1993; Brichard et al., 1993; Jerome et al., 1991). Autologous tumor reactive CTLs can be generated from lymphocytes infiltrating ovarian malignant ascites (Ioannides et al., 1991), and overexpressed proteins, such as HER-2, may be targets for CTL recognition (Ioannides et al., 1992).

T-cells play an important role in tumor regression in most murine tumor models. Tumor infiltrating lymphocytes (TIL) that recognize unique cancer antigens can be isolated from many murine tumors. The adoptive transfer of these TIL in addition to interleukin-2 can mediate the regression of established lung and liver metastases (Rosenberg et al., 1986). In addition, the secretion of IFN-γ by injected TIL significantly correlates with in vivo regression of murine tumors suggesting activation of T-cells by the tumor antigens (Barth et al., 1991). The known ability of TIL to mediate the regression of metastatic cancer in 35 to 40% of melanoma patients when adoptively transferred into patients with metastatic melanoma attests to the clinical importance of the antigens recognized (Rosenberg et al., 1988; Rosenberg, 1992).

Strong evidence that an immune response to cancer exists in humans is provided by the existence of tumor reactive lymphocytes within melanoma deposits. These lymphocytes, when isolated, are capable of recognizing specific tumor antigens on autologous and allogeneic melanomas in an MHC restricted fashion. (Itoh et al., 1986; Muul et al., 1987; Topalian et al., 1989; Darrow et al., 1989; Hom et al., 1991; Kawakami et al., 1992; Hom et al., 1993; O'Neil et al., 1993). TIL from patients with metastatic melanoma recognize shared antigens including melanocyte-melanoma lineage specific tissue antigens in vitro (Kawakami et al., 1993; Anichini et al. 1993). Anti-melanoma T-cells appear to be enriched in TIL, probably as a consequence of clonal expansion and accumulation at the tumor site in vivo (Sensi et al., 1993). The transduction of T-cells with a variety of genes, such as cytokines, has been demonstrated. T-cells have been shown to express foreign gene products. (Blaese, 1993; Hwu et al., 1993; Culver et al., 1991) The fact that individuals mount cellular and humoral responses against tumor associated antigens suggests that identification and characterization of additional tumor antigens is important for immunotherapy of patients with cancer.

T-cell receptors on $CD8^+$ T-cells recognize a complex consisting of an antigenic peptide (9-10 amino acids for HLA-A2), β2 microglobulin and class I major histocompatibility complex (MHC) heavy chain (HLA-A, B, C, in humans). Peptides generated by digestion of endogenously synthesized proteins are transported into the endoplastic reticulum, bound to class I MHC heavy chain and β2 microglobulin, and finally expressed in the cell surface in the groove of the class I MHC molecule.

Information on epitopes of self-proteins recognized in the context of MHC Class I molecules remain limited, despite a few attempts to identify epitopes capable of in vitro priming and Ag-specific expansion of human CTLs. For example, peptide epitopes have been proposed which are likely candidates for binding on particular MHC Class I Ag (Falk et al., 1991), and some studies have attempted to define peptide epitopes which bind MHC Class I antigens.

Synthetic peptides have been shown to be a useful tool for T-cell epitope mapping. However in vivo and in vitro priming of specific CTLs has encountered difficulties (Alexander et al., 1991; Schild et al., 1991; Carbone et al., 1988). It is generally considered that in vitro CTL priming cannot necessarily be achieved with peptide alone, and in fact, a high antigen density is thought to be required for peptide priming (Alexander et al., 1991). Even in the limited instances when specific priming was achieved, APC or stimulators were also required at high densities (Alexander et al., 1991).

Short synthetic peptides have been used either as target antigens for epitope mapping or for induction of in vitro primary and secondary CTL responses to viral and parasitic Ags (Bednarek et al., 1991; Gammon et al., 1992; Schmidt et al., 1992; Kos and Müllbacher, 1992; Hill et al., 1992). Unfortunately, these studies failed to show the ability of proto-oncogene peptide analogs to stimulate in vitro human CTLs to lyse tumors endogenously expressing these antigens.

Identification of tumor antigens (Ag) and of specific epitopes on these Ag recognized by cytotoxic T-lymphocytes enables the development of tumor vaccines (for review of tumor antigens, see Rosenberg (2000), incorporated by reference herein). Tumor Ag are weak or partial agonists for activation of low-avidity (low-affinity) CTL. Attempts to activate CTL by increasing the affinity of peptide for MHC (by modifications in the anchor residues) has produced mixed successes even with powerful APC (dendritic cells, DC) and added B7 costimulation. Some of the resulting cross-reactive CTL recognized tumors with lower affinity than CTL induced by wild type Ag.

The limited ability of anchor-fixed immunogens to induce and expand high-affinity CTL raises the need for alternative approaches for CTL induction. One approach to this question is to design immunogens which activate "high-affinity" CTL from the existent pool of responders. In human tumor immunology, this approach has been successful in some instances. However, high-affinity CTL are expected to be more sensitive to silencing by elimination (e.g apoptosis) or by anergy (unresponsiveness or diminished reactivity to a specific antigen).